INCREASING INCIDENCE OF IMMUNE MEDIATED NECROTIZING MYOPATHY – SINGLE CENTRE EXPERIENCE

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ABSTRACT:

Objectives. Immune mediated necrotizing myopathy (IMNM) is characterized by predominant presence of necrotic muscle fibres in muscle biopsy and variable response to immunosuppressive treatment. We have analysed the incidence of IMNM in our centre over the last ten years and explored the role of statins as possible causative agents.

Methods. A retrospective evaluation of muscle biopsy results, clinical and laboratory data including antibody associations of all patients with IIM newly diagnosed between 2004 and June 2014 was performed. Available sera were tested for the presence of anti-HMGCR autoantibodies.

Results. Out of 357 biopsied patients, 233 fulfilled criteria for inflammatory/immune mediated myopathy, including 27 (11.6%) classified as IMNM. There were no patients with IMNM diagnosed between 2004 and 2007; subsequently 2-3 cases of IMNM per year were seen during the period of 2008 to 2011 with a substantial increase to 18 cases (66.6% of all IMNM biopsies) in 2012-2014. Thirteen out of 27 patients (48%) had a history of statin use; eleven (85%) of which had positive anti-HMGCR antibodies. There was no IMNM patient without a history of statin use who was anti-HMGCR antibody positive.

Conclusions. Our data show an increasing incidence of IMNM, which is mainly accounted for by anti-HMGCR positive IMNM associated with use of statins.

KEY WORDS: myositis, necrotizing myopathy, muscle biopsy, anti-HMGCR autoantibodies

INTRODUCTION:

Immune mediated necrotizing myopathy (IMNM) is a relatively newly recognized category of idiopathic inflammatory myopathy (IIM). It is characterized by predominant presence of necrotic muscle fibres with minimal or no inflammatory infiltrates in muscle biopsy and variable degree of response to immunosuppressive treatment.[1-3] IMNM itself is a heterogeneous group – it is often associated with the presence of autoantibodies, e.g. anti-SRP or anti-HMGCR, each representing about 3-6% of IIMs.[3, 4] The diagnosis of IMNM seems to be getting more frequent in our centre, therefore we decided to retrospectively analyse the annual incidence of IMNM since 2004 and to compare it with the incidence of other forms of myositis. We have also investigated a possible contribution of statins as causative agents.

PATIENTS AND METHODS:

Muscle biopsies, as well as clinical and laboratory data, of all patients who were evaluated at the Institute of Rheumatology between January 2004 and June 2014 for suspicion of IIM were retrospectively reviewed. This time period was selected because since 2004 all muscle biopsies were performed in the same hospital and were read by a single expert pathologist (JZ).[5] Patients who fulfilled Bohan and Peter myositis criteria [6, 7] or ENMC [1] criteria for necrotizing myopathy or Griggs criteria for IBM [8] were included in the analysis as cases of inflammatory/immune mediated myopathy. All patients signed an informed consent and the study was approved by local ethics committee.

Muscle samples were obtained by open biopsy from quadriceps muscle (mostly lateral vastus muscle) under a local anaesthesia. Isopentane frozen samples were examined using haematoxylin–eosin staining and a spectrum of histochemical and immunohistochemistry reactions as well as by electron microscopy.[9] Biopsy results were subcategorized, blinded to the clinical diagnosis, according to the report of the 119th ENMC (European Neuromuscular Centre) workshop [1] as: immune mediated necrotizing myopathy (IMNM), polymyositis (PM), dermatomyositis (DM), non-specific myositis (NS-M) and inclusion body myositis (IBM). Biopsies with significant pathologies, but not consistent with a single diagnostic category were labelled as non-classifiable (NC). Biopsies with no pathological changes or with mild nonspecific abnormalities were classified as normal. Only good quality muscle biopsies that provided sufficient amount of tissue were considered for the study.

Personal history, clinical data, laboratory results and data regarding environmental exposure were obtained from patient database and/or hospital records.

Anti-HMGCR autoantibodies were measured by ELISA in 218 patients and in 62 healthy controls. Sera were tested without knowledge of clinical details. Briefly, 96 well plates were coated with 1.0

µl/ml HMGCR antigen (Sigma-Aldrich, St. Louis, Missouri) in PBS overnight at 4°C prior to being blocked in PBS-0.1%BSA-0.1%Tween at room temperature for 2 hours. Serum samples were diluted 1/200 in PBS-0.1%Tween and were added to the plate in duplicate for 2 hours at 4°C. Plates were washed three times in PBS-0.1%Tween prior to the addition of 1:30000 Goat anti-Human IgG (Sigma-Aldrich) at 4°C for 30 min. Plates were washed three times in PBS-0.1%Tween and incubated in TMB Substrate Solution (Sigma-Aldrich) at 4°C for 10 minutes. Reactions were stopped with 1M H2SO4 and plates were read at 450nm. Negative cut-offs were calculated from the mean OD + three standard deviation of healthy controls. All positive samples were confirmed on at least two repeated ELISAs.

Autoantibody profiles of IIM patients were determined during routine diagnostic workup using indirect immunofluorescence to screen for antinuclear antibodies (ANA) and anti-dsDNA (Immuno Concepts, Sacramento, USA), line immuno assay (Imtec Human, Wiesbaden, Germany) and myositis-westernblot (Euroimmun, Lübeck, Germany) for detection of individual autoantibodies directed against Jo-1, Mi-2, Ku, PM-Scl, PM-Scl75, PM-Scl100, PL-7, PL-12, EJ, OJ, SRP, Ro, Ro52, La, Scl-70, and U1-RNP antigens. In-house made ^{35}S radioimmunoprecipitation [10] was used to confirm the results and to detect autoantibodies not captured using commercial assays (antibodies to: TIF-1γ, MDA5, NXP2, Zo, EIF3, RNAP I, RNAP II, and RNAP III). Rheumatoid factors (RF) were detected using a particle-agglutination assay (Fujirebio Inc., Tokyo, Japan); and an ELISA test for anti-CCP (Test – Line Clinical Diagnostics, Brno, Czech Republic) was used to detect antibodies against anti-citrullinated peptides (ACPA).

Data on the country-wide statin use were obtained from publicly available information at the Czech State Institute for Drug Control web- page.[11, 12]

Demographics, clinical characteristics, and results are presented as descriptive statistics. Categorical data were analysed by χ^2-test and Fisher's exact test. We used GraphPad Prism 5 (GraphPad Software, La Jolla, California) for statistical analysis.

RESULTS:

Out of the 357 patients who had muscle biopsy performed during the period 2004 - 2014, 233 patients (171 [73.4%] females; mean age 55.45±13.66 years) were diagnosed with inflammatory/immune mediated myopathy. Muscle biopsy results evaluated according to the 119th ENMC workshop report [1] were classified as PM in 65 (27.9%), DM in 90 (38.6%), IMNM in 27 (11.6%), NS-M in 5 (2.1%), and IBM in 6 (2.6%) cases. Five (2.1%) biopsies were non-classifiable and 35 (15.0%) were normal based on the above mentioned criteria.

Eight patients had overlap syndromes: five with systemic lupus erythematosus, and three with systemic sclerosis. Two patients fulfilled criteria for mixed connective tissue disease (MCTD).[13]

Twenty-seven patients were diagnosed with cancer associated myositis, defined as occurrence of cancer within three years of IIM diagnosis, with breast and ovarian cancer being the most frequent tumours in 7 (26%) and 5 cases (18%) respectively. IMNM based on the clinical and histology ENMC criteria was diagnosed in 27 patients; out of which one had an overlap with rheumatoid arthritis and one had skin melanoma.

Biopsy results, autoantibody profiles, as well as selected clinical and environmental characteristics of the patients with necrotic biopsies are shown in Table 1. Apart from anti-HMGCR autoantibodies and previous use of statins, we have not found an association with any of the demographic, clinical, laboratory, or environmental factors that have been analyzed.

Incidence

Overall, 27 (11.6%) cases were histologically classified as necrotizing myopathy and diagnosed with IMNM based on the ENMC criteria. There were no necrotizing myopathies diagnosed between 2004 and 2007. Subsequently 2-3 cases of IMNM per year were seen during the period of 2008 to 2011 with a substantial increase to 10 cases in the year 2012 (43.5% of all NM biopsies and 35.8% of biopsies performed that year), which is significantly more than during the 2004-2011 period (χ^2 [df1] =54.124, $p<0.0001$). This trend was confirmed in the following 18 months (January 2013 to June 2014) (Figure 1): eight identified necrotizing myopathies also exceeds the incidence observed during the years 2004-2011 (χ^2 [df1] =30.268, $p<0.0001$). The rapid increase of necrotizing myopathy incidence in the recent two and a half years (2012 – June 2014) represents a significant change compared with previous years (χ^2 [df1] =82.460, $p<0.0001$).

Most biopsies of patients with IMNM displayed prominent muscle fibre necrosis without any inflammatory infiltrates. There were 10 biopsies with scarce lymphocytes, that stained positively for CD8 in 6 cases and for CD20 in one case. In the remaining three biopsies lymphocytes were not present on the slides used for immunostaining and therefore could not be typed. There were no detectable differences in biopsy pattern with respect to the presence or absence of anti-HMGCR antibodies.

Autoantibodies

Out of 217 serum samples available, anti-HMGCR autoantibodies were found in 15 (6.9%); 16 patients who were not tested neither had necrotic finding on biopsy nor used statins. Eleven out of the fifteen anti-HMGCR positive patients had necrotic histology on biopsy and four were classified as PM based on a classical finding of invasion of inflammatory cells into muscle fibres in three of them and inflammatory infiltration surrounding muscle fibres without invasion in one case.

Four IMNM patients were positive for anti-SRP antibodies, two were anti-Jo-1 positive, two were anti-Ro/La positive, one was anti-Ku positive, one had isolated anti-Ro52 positivity and four were ANA positive with no identifiable specific autoantibody. Anti-CCP antibodies, considered highly specific for rheumatoid arthritis (RA), were detected in two IMNM patients; one of them had an overlap with RA (Patient No.13). Anti-HMGCR autoantibodies were found in 11 patients, overlapping with other autoantibodies in two patients only (anti-CCP autoantibodies and ANA). There were no differences in muscle strength, presence of myalgia, or serum CK levels between anti-HMGCR positive and negative patients.

Statin use

Out of the total of 233 patients with IIM, 36 (15.5%) had a history of prior statin use (data were not available for 2 individuals). Among the 36 statin users, 15 (41.7 %) developed anti-HMGCR antibodies and 13 (36.1 %) presented with necrotising myopathy in the biopsy. Eleven out of the 13 patients with IMNM and a history of statin use were positive for anti-HMGCR antibodies. The two IMNM patients exposed to statins without anti-HMGCR antibodies were anti-Ro52 and ANA positive respecitvely. All 15 anti-HMGCR positive patients were treated by statins in the past (100%) whereas only 21 out of the remaining 202 (10.4%) anti-HMGCR negative IIM patients had a history of statin use ($p<0.0001$). Thirteen out of the 27 IMNM patients were statin users (48.1 %) whereas only 23 among 204 (11.3%) non-IMNM patients were exposed to statins ($p<0.0001$).
There is a strong association in our cohort between statin use and presence of anti-HMGCR positive necrotising myopathy since 11 out of 15 anti-HMGCR positive patients who used statins developed IMNM. There were only 4 anti-HMGCR positive patients with history of statin exposure who did not have necrotising myopathy. These 4 patients showed classical polymyositis pattern on biopsy.
These findings suggest a strong association of statin use with both the development of anti-HMGCR antibodies and with necrotising myopathy.

Atorvastatin, simvastatin, fluvastatin and rosuvastatin were used by 22 (61%), 9 (25%), 2 (5.5%), and 2 (5.5%) patients respectively. Four patients switched different statins and in five patients (13.8%) the specific statin used was not known. Individual drugs and details on their use are summarized in Table 1.
None of the IMNM patients was on statins at the time of biopsy (mean time between statin discontinuation and muscle biopsy was 16.2±28.8 months; range 2 weeks to 8 years). In the group of non-IMNM patients, five had biopsy performed while using statins and in two individuals the exact date of statin discontinuation was not known. In all the other non-IMNM patients the mean time between stopping statins and muscle biopsy was 7.5±15.2 months (range 2 weeks to 5 years; ns).
Three patients were on a combination of statin and fibrate: two of them had classical polymyositis

pattern in muscle biopsy with one of them being anti-HMGCR positive, the third patient had normal biopsy findings and was anti-HMGCR negative.
Patients using statins were older than non-users (64.5±7.7 years vs. 53.8±13.9 years; $p<0.0001$ for whole group and 67.6±6.6 years vs. 45.9±18.1 years; $p=0.0004$ for IMNM cohort).

DISCUSSION:

We report a significantly increasing incidence of immune mediated necrotizing myopathy among patients evaluated for idiopathic inflammatory myopathy in our centre over the past ten years. No IMNM cases were seen in the years 2004-2007, first patients with IMNM started to appear during the 2008 – 2011 period with a sharp increase in 2012, and this higher frequency has been maintained in 18 subsequent months. Referral pattern remained unchanged during the whole period of our study. We have evaluated all biopsies performed since 2004 in our centre, irrespective of the final diagnosis and all biopsies were taken and processed in the same way at the same department and read by a single experienced pathologist using a pre-specified protocol for the recording of pathological findings. Therefore we believe the number of IMNM cases is truly increasing over time and the results are not influenced by recent interest in necrotizing myopathies.

Based on our results it seems that, for the most part, the statin-induced IMNM is responsible for the increasing incidence. Ten out of 18 patients with IMNM seen in 2012-2014 were treated with statins and all but one had anti-HMGCR antibodies, thus confirming the likely role of statins in the pathogenesis of the disease. In addition two other patients seen in 2011were treated with statins and were anti-HMGCR positive. Only two statin users among IMNM patients did not have the anti-HMGCR antibodies. Moreover, anti-HMGCR antibodies were not found in any statin non-user either with IMNM, or with another subtype of IIM. This strong association of anti-HMGCR antibodies and statin use among IMNM patients is remarkable and confirms previous results reported in patients from Johns Hopkins University.[15]

More than eighty per cent of patients with statin-associated IMNM from our cohort used atorvastatin. This finding probably reflects the fact that atorvastatin has been the most frequently prescribed statin in the Czech Republic since 2006 and is becoming ever more popular (Figure 2).[11, 12] The disease is, however, not limited to atorvastatin users and other statins are implicated too. The average duration of statin use prior to the onset of symptoms was 2.67 years (range 2 months to 6.5 years) in accordance with previous findings.[16]

In summary, we describe an increased incidence of necrotising myopathy in recent years. Almost half of the cases are anti-HMGCR positive IMNM patients. Statins are most likely responsible given the striking association with the presence of anti-HMGCR positivity.

KEY MESSAGES:

Necrotizing myopathy increases in incidence.

Increase in the incidence of immune mediated necrotizing myopathy is statin-associated.

CONFLICT OF INTEREST STATEMENT

All authors declare no conflicts of interest.

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Table 1. Characteristics of patients with necrotizing myopathy in the muscle biopsy.

Patient No.	Gender/Age (years)	Biopsy date M/Y		Autoantibodies to:	Statin use	Statin type	Duration of statin use (years)	Muscle strength (%)[a]	Myalgia	Maximal serum CK level (μkat/L)[b]	Systemic and organ involvement
1	M/24	8/2008		SRP	N	-	-	62.5	N	37	-
2	M/61	9/2008		Ro, La	N	-	-	74.3	Y	75	Fever
3	M/31	11/2009		Jo-1	N	-	-	97.5	N	111	Fever, arthritis
4	F/62	11/2009		ANA	N	-	-	73.8	N	16	-
5	F/55	1/2010		Ro52	Y	S	3	56.3	Y	0.4	-
6	F/54*	5/2010		ANA	N	-	-	88.6	N	38	-
7	M/19	12/2010		Jo-1	N	-	-	88.8	Y	279	Fever, arthritis
8	M/55	4/2011		HMGCR	Y	A	UN	76.3	Y	211	-
9	F/67	10/2011		HMGCR	Y	S/A	3.75	73.8	N	172	-
10	F/64	2/2012		HMGCR	Y	A/R	6.5	66.3	Y	229	-
11	F/76	2/2012		HMGCR	Y	S/A	2	67.5	N	158	-
12	M/70	3/2012		HMGCR	Y	A	3	67.5	Y	157	-
13	F/43§	3/2012		CCP, ANA	N	-	-	67.5	N	138	Arthritis§
14	F/67	7/2012		SRP	N	-	-	60	N	366	-
15	F/56	9/2012		SRP	N	-	-	67.5	N	103	-
16	F/67	10/2012		HMGCR	Y	A	3	91.3	N	112	-
17	F/57	10/2012		N	N	-	-	83.8	N	15	-
18	F/65	11/2012		HMGCR, CCP	Y	F	0.5	70	N	68	-
19	F/19	12/2012		N	N	-	-	56.3	Y	200	-
20	M/73	1/2013		HMGCR	Y	A	UN	63.8	N	80	-
21	F/72	2/2013		HMGCR	Y	A	1yr	62.5	N	24	-
22	F/77	7/2013		ANA	Y	A	UN	UN	N	82	-
23	F/67	9/2013		HMGCR	Y	A	4	UN	N	94	-
24	M/66	1/2014		HMGCR	Y	A	0.2	UN	Y	27	-
25	M/49	4/2014		ANA, Ro60, La	N	-	-	97.5	Y	128	Arthritis, Raynau

											d's phenomenon
26	M/65	6/2014		SRP	N	-	-	70	N	139	-
27	M/28 †	6/2014		ANA, Ku	N	-	-	85	N	70	-

M/F – male/female; Y/N – yes, no;

S – simvastatin, A – atorvastatin, R – rosuvastatin, F– fluvastatin. UN – unknown

[a]Muscle strength shown as a percentage of maximal strength derived from 8-muscle Manual Muscle Test (MMT8).[14]

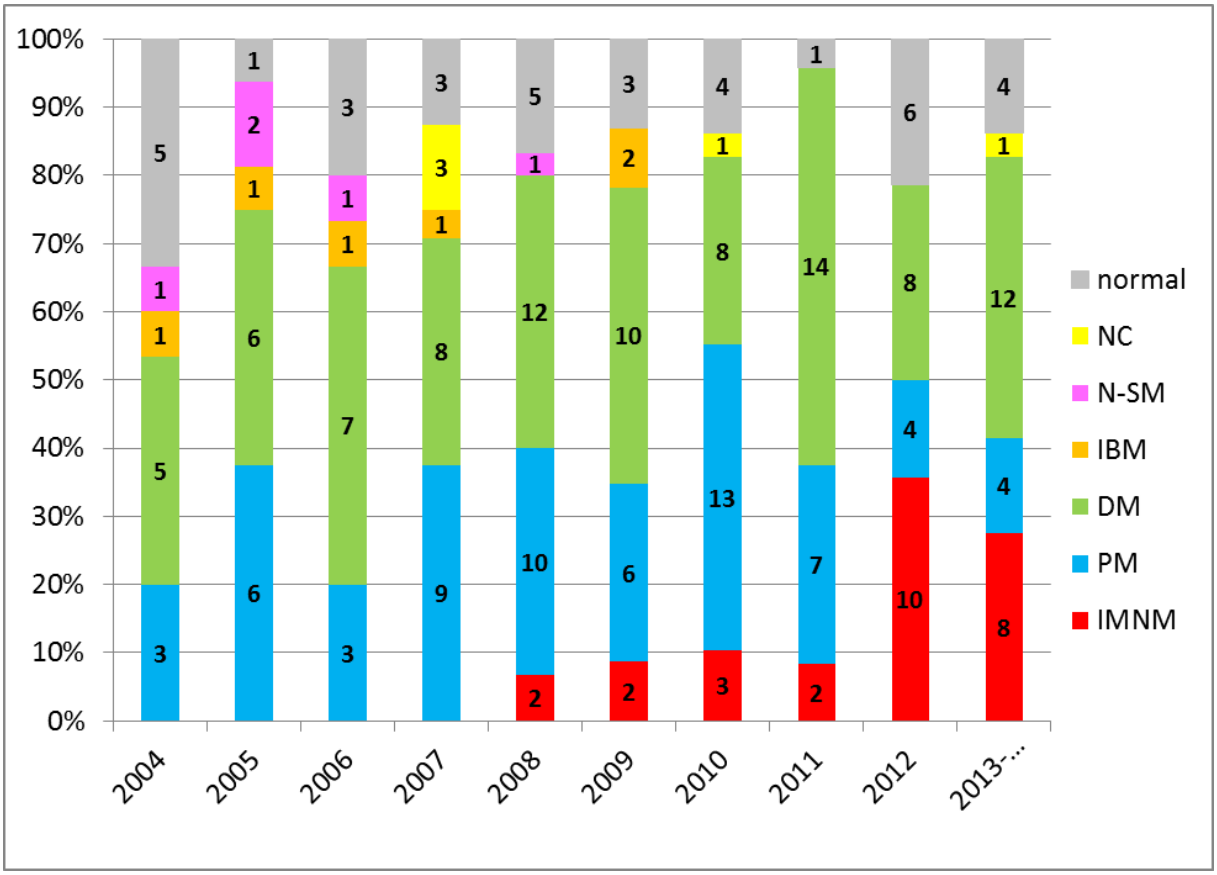
[b]Normal range in our laboratory 0.05-2.42 μkat/L

[*]Skin melanoma

[§]Overlap syndrome with rheumatoid arthritis

[†] Overlap syndrome with systemic lupus erythematosus

Figure 1. Percentage distribution of biopsy results in individual years.

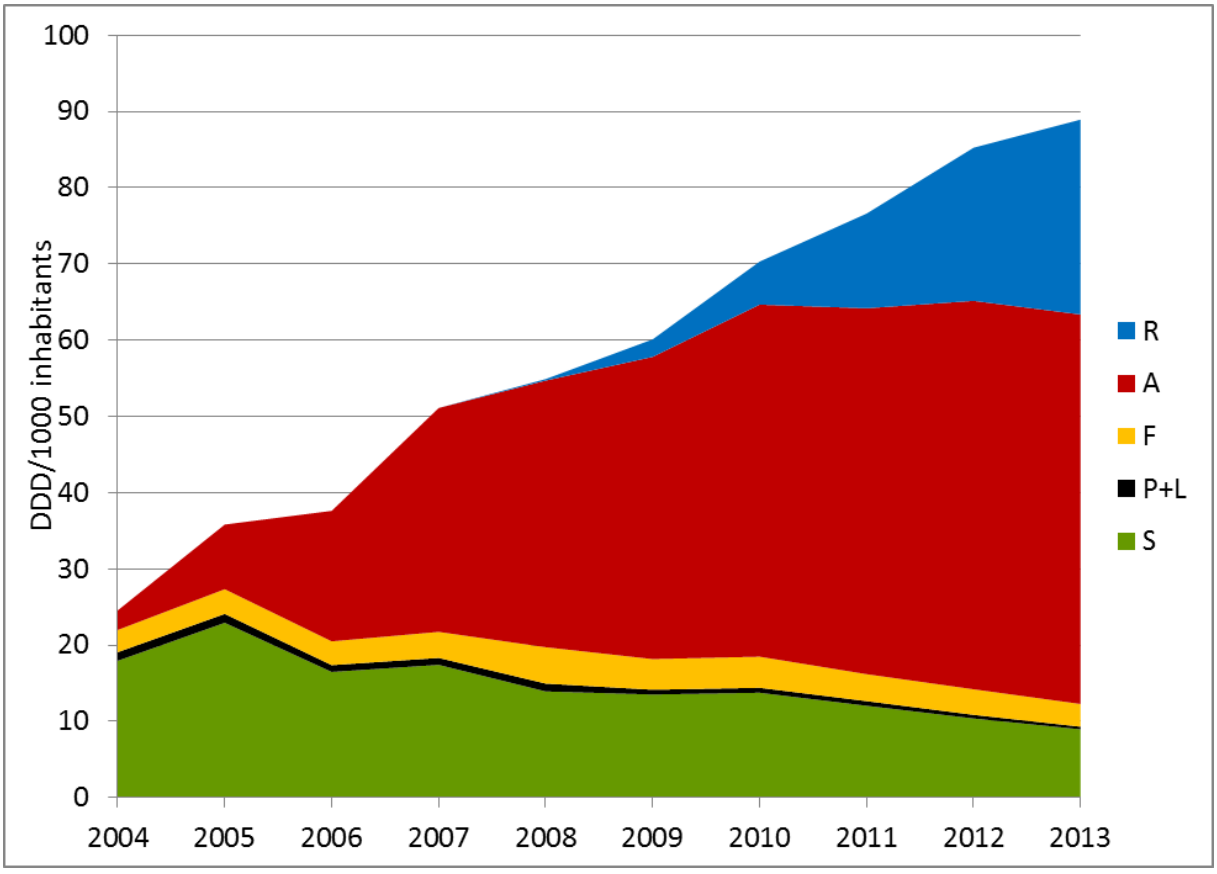


Results: NC – non-classifiable; IBM – inclusion body myositis; N-SM – non-specific myositis; DM – dermatomyositis; PM – polymyositis, IMNM – immune mediated necrotizing myopathy

* Data from January 2013 to June 2014.

Numbers in boxes indicate absolute numbers of biopsy results.

Figure 2. Statin use in the Czech Republic between 2004 and 2013.



R – rosuvastatin; A – atorvastatin; F – fluvastatin; P+L – pravastatin and lovastatin (pooled); S – simvastatin. Data shown as DDD (Defined Daily Doses) per 1 000 inhabitants according to WHO methods.